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4-Colour flow cytometry demonstrates CD3+, CD4+ and CD8+ cells which produce TH1 type cytokines in Helicobacter pylori gastritis.
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Adoptive transfer of Helicobacter-specific TH1 or TH2 cells exacerbates Helicobacter-associated gastritis, but only TH2 cells reduce the magnitude of infection.
AUTHOR: Mohammadi M; Czinn S; Redline R; Nedrud J
AUTHOR ADDRESS: Case Western Reserve Univ., Cleveland, OH**USA
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15
Is the Th1/Th2 lymphocyte balance upset by *Helicobacter pylori* infection?

P. B. ERNST, V. E. REYES, W. R. GOURLEY, H. HÄBERLE and K. B. BAMFORD

INTRODUCTION

Since *H. pylori* became recognized as a gastric pathogen, many people have felt that antibiotics would expedite the eradication of, or at least the decrease in, gastric diseases associated with this infection. However, history reminds us that antibiotics have been relatively ineffective at eradicating other bacterial infections. In the case of *H. pylori* the infection is widespread on a global basis. Although the rate of infection with *H. pylori* is slow, our understanding of the epidemiology of the spread of this pathogen is incomplete, thereby limiting the success of interventions to decrease the incidence of infection. Furthermore, antibiotic regimens may be too expensive for some individuals or societies, and resistance to antibiotics is emerging. Thus, it is essential to understand the immunopathogenesis of gastric disease associated with *H. pylori* infection and to counter these processes through vaccines that may shift the host response away from chronic inflammation and tissue destruction towards effective immunity. This review will summarize the means by which mucosal immune responses can confer protection to luminal flora without inducing disease. In addition, the current understanding of T-cell biology in *H. pylori* infection will be examined and contrasted with the T-cell responses that may be induced by the vaccines for *H. pylori* that are currently in the development stage.

GOAL OF MUCOSAL IMMUNITY

Mucosal immune responses in the digestive tract have evolved such that they are capable of conferring protection without inducing excessive amounts of inflammation. Current dogma suggests that secretion of IgA into the lumen is largely responsible for achieving immunity. This is based on the fact that more than 90%

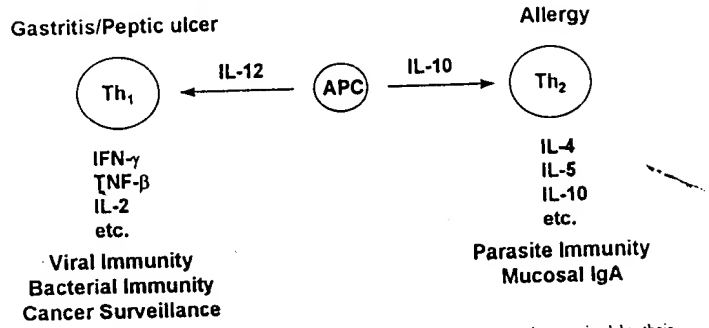

Figure 1 Characterization of Th1 and Th2 cells. Th1 and Th2 cells are characterized by their cytokine profile. After exposure to IL-12, Th1 cells are selected and produce IFNγ, TNFβ and IL-2 that can combine to increase cell-mediated immunity. They also can increase complement-fixing IgG and enhance phagocytosis of opsonized bacteria. However, Th2 cells have a decided advantage at inducing mucosal immune responses including IgA. Either subset can also contribute to disease. For example, Th2 cells are implicated in the pathogenesis of allergies and Th1 could contribute to epithelial damage in the stomach

of the B cells and plasma cells in healthy gastrointestinal tissue produce IgA. Moreover, IgA is secreted in the digestive tract as a dimer – a structure that permits it to be transported across the epithelium and into the lumen. IgA has been shown to be capable of neutralizing bacteria or their toxins. Despite this, IgA deficiency is the most common immunodeficiency in humans, and these individuals have little disadvantage in their ability to prevent infections. Thus, other mechanisms may contribute to protection, but IgA is likely to play a significant role.

HELPER T CELL HETEROGENEITY

Immunological effector mechanisms may be driven by antigen but the magnitude and type of immune response that develops is largely dictated by cytokines. Helper T cells can be functionally divided into subsets based on their cytokine profile. Through the production of interferon-γ (IFNγ), TNFα and IL-2, Th1 cells select for a rather specific panel of immune responses including cell-mediated immunity, while Th2 cells preferentially regulate mucosal IgA responses through the production of TGFβ, IL-4, IL-5, IL-6 and IL-10[1] (Figure 1). Since naive T cells retain the potential to differentiate into either subset, most antigens probably induce a mixture of T-cell responses. However, over time, some pathogens that are associated with chronic infection favour a response that is predominantly either Th1 or Th2 in nature. Both subsets have been shown to play a pivotal role in immunity and both also have been implicated in the immunopathogenesis of disease[2]. Thus, in order to maintain health the host must strike the correct balance between these subsets.

87. **Eaton, K. A., Dewhirst, F. E., Radin, M. J., Fox, J. G., Paster, B. J., Krakowka, S., and Morgan, D.R.,** *Helicobacter acinonyx* sp. nov., a new species of *Helicobacter* isolated from cheetahs with gastritis, *Int. J. Syst. Bact.*, in press.
88. **Eaton, K. A., Radin, M. J., Kramer, L., Wack, R., Sherding, R., Krakowka, S., Fox, J. G., and Morgan, D. R.,** Epizootic gastritis in cheetahs associated with gastric spiral bacilli, *Vet. Pathol.*, in press.
89. **Czinn, S. J. and Nedrud, J. G.,** Oral immunization against *Helicobacter pylori*, *Infect Immun.*, 59, 2359, 1991.
90. **Chen, M., Lee, A., and Hazell, S.,** Immunization against gastric helicobacter infection in a mouse *(Helicobacter felis)* model, *Lancet*, 1120, 1992.
91. **Czinn, S., Cai, A., and Nedrud, J.,** Oral immunization protects germ-free mice against infection from *Helicobacter felis*, *Gastroenterology*, A331, 102, 1992.
92. **Eaton, K. A. and Krakowka, S.,** Chronic active gastritis due to *Helicobacter pylori* in immunized gnotobiotic piglets, *Gastroenterology*, in press.

Chapter 25

OVERVIEW OF *HELICOBACTER PYLORI* GASTRITIS, PEPTIC ULCER, AND GASTRIC CANCER AND THE POSSIBLE DEVELOPMENT OF AN *H. PYLORI* VACCINE

C. Stewart Goodwin

TABLE OF CONTENTS

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I. CONFUSIONS AND A UNIFYING CONCEPT

During the first decade since the discovery of *H. pylori*, arguments about its significance have been confused, first by a failure to distinguish between upper gut symptoms and their relief on the one hand, and histological gastritis lesions due to *H. pylori* on the other. Such lesions occur in asymptomatic patients and in endoscopically "normal" stomachs, but may still lead on to peptic ulcer or gastric cancer. Second, in developed countries 50% of patients with upper gut symptoms do not have *H. pylori* infection, and the cause(s) of these symptoms could certainly be significant in *H. pylori*-positive patients; until these causes are fully elucidated, assessment of symptoms due to *H. pylori* will be confused. Third, many patients with *H. pylori* inflammation seem to have minimal or no symptoms. Therefore, non-ulcer dyspepsia is a confused subject (see Chapters 4 and 23). However, with a careful clinical history and examination, I have found that some such patients treated with antibiotics have been very grateful that their symptoms have gone.

A unifying concept of the relationship between *H. pylori* and the immune system has been delineated by Blaser.[1] He likened *H. pylori* infection to lepromatous leprosy, with a balance between the body's *inability* to eliminate the bacteria, and the body's *ability* to suppress harmful tissue reaction. In borderline and tuberculoid leprosy there are fewer bacteria, but fierce inflammation in nerves leads to paralysis. In *H. pylori* infection, heat shock protein homologues stimulate autoimmunity, and when there is continuous polymorph inflammation, this will lead sooner to gastric atrophy, and in some patients to cancer. In both diseases there is evidence of decreased antigen response to the pathogen, and the dominant T cells are of the suppressor type. Blaser suggested that *H. pylori* obtains nutrients by inflammatory disruption of mucosal barriers, but it is in the interest of both pathogen and host to restrict inflammation to postpone atrophy. Another useful concept, suggested by Oi et al. in 1959,[2] was that peptic ulcer is an "ulcer of junction" at the point of transition from one type of epithelium, which may be relevant for both duodenal and gastric ulceration. In Chapter 3, the probable role of some vascular factor, in addition to *H. pylori* and acid and pepsin,[3] is significantly made. The pathogenesis of gastric ulcer (GU) is certainly less clear than duodenal ulcer; although gastritis is a common lesion, GU is infrequent. The effect of age of acquisition of *H. pylori* on subsequent pathology is shown in Figure 1.

II. PATHOGENESIS OF GASTRITIS DUE TO *H. PYLORI*

A. ACHIEVEMENT OF PRIMARY INFECTION

The alternative routes for *H. pylori* infection, of fecally contaminated food or water or oral-oral contact are fully discussed in Chapter 6. Significantly, *H. pylori* DNA has been detected by PCR in the feces of 26 out of 29 infected patients;[3a] although this DNA could come from dead organisms, the distance

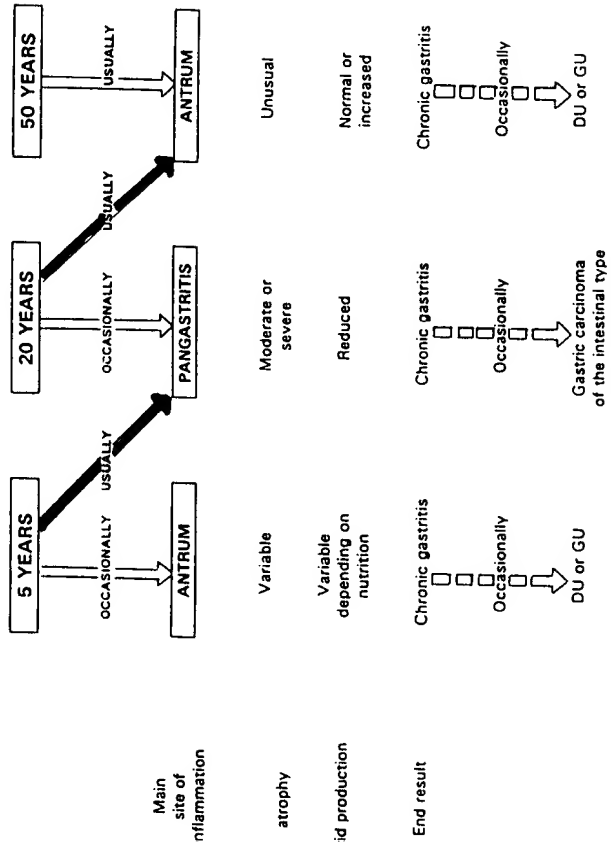

FIGURE 1. The influence of the age of acquisition of *H. pylori* gastritis on subsequent pathology.

from the stomach and the high percentage suggest the bacteria could be alive and infectious. Fecal transmission was likely in Chile, where the incidence of *H. pylori* was unexpectedly found to be higher in those who ate uncooked vegetables (irrigated with contaminated water) than those who ate only cooked vegetables.[3b] The coccal form of *H. pylori* is likely to be present when conditions are unfavorable for the organism. Whether this coccal form can rapidly develop in the stomach into an active bacterial form which can invade the mucus and adhere is theoretically doubtful. Work must be done in animal models. After the primary ingestion of *H. pylori*, various factors aid its success in entering the area under the mucus, superficial to the gastric mucosa. Acid destruction of the organism is withstood by its urease; urease-deficient mutants are sensitive to acid and exhibit no cytotoxicity.[4] The urease enzymes of gastric helicobacters — *H. pylori*, *H. mustelae*, and *H. felis* — have two pH optima, including one at acid pH,[5,6] but the latter is missing from the helicobacters that colonize the lower gut, such as *H. muridarum*.[6] It has been suggested that the possession of such a urease is a prerequisite for the ability to colonize the stomach. Second, *H. pylori* can pass through thick mucus much more easily than rod-shaped bacteria, and this is mentioned in Chapter 2. The surface of the organism may assist in passage through the mucus and adherence. Hydrophobic interaction chromatography experiments indicate that the outer layer of *H. pylori* is hydrophobic.[7] However, other methods show that the outer layer of *H. pylori* is hydrophilic.[8] It is probable, therefore, that there are hydrophobic domains in an overall hydrophilic surface. The organism is able to change the structure of mucin, which could aid its penetration through the stomach mucin layer.[9] The bacteria must maintain their site in the stomach by actively adhering to the gastric mucosa or moving in the mucus, because there is a continual passage of mucus down the stomach and into the duodenum. Adherence mechanisms in the stomach have been discussed in Chapter 12. These could be considerable importance in relation to the development of a vaccine (see below).

B. INFLAMMATION IN ASSOCIATION WITH *H. PYLORI*

The intensity of this inflammation may be due to different factors, including autoimmunity, suggested by the fact that cross-reacting antibodies with gastric mucosa are found in patients with *H. pylori* infection.[10] Monoclonal antibodies against *H. pylori* cross-react with the cells of the human gastric mucosa and also with murine gastric epithelial cells. A strong reaction against autologous mucosa was evident in the sera of mice immunized with *H. pylori* but not with other bacteria. Seropositivity against *H. pylori* strongly correlated with the presence of autoantibodies against human antral gastric mucosa. This activity was lost after absorption of the serum with *H. pylori* but not with other Gram-negative bacteria. The antibodies in the mouse and in the human did not react with other segments of the gastrointestinal tract. Also, mice bearing hybridomas secreting one of the cross-reacting antibodies had histopathological abnormalities in their stomachs. Heat-shock protein homologs of *H. pylori* are

discussed in Section VII. Interestingly, in AIDS patients with *H. pylori* infection, there is considerably less inflammation than in uninfected people.[11] A component of the inflammation may require T cells, which are greatly reduced in AIDS.

Also, Karttunen[12] reported that a whole, inactivated *H. pylori* preparation stimulated blood lymphocytes from both antibody-positive and antibody-negative subjects, but the antibody-positive subjects tended to have lower proliferation responses. *H. pylori*-induced DNA synthesis was lower in the antibody/bacterium-positive subjects. The numbers of HLA-DR$^+$ and IL-2R$^+$ T cells increased in cultures with *H. pylori*, but the respective CD8 subsets were increased only in the antibody-positive subjects. The confirmed decrease in proliferation in the antibody-positive subjects may involve CD8 cell activation. Other aspects of cellular immunity are discussed in Chapter 15.

C. DAMAGE TO GASTRIC MUCOSAL CELLS

New Zealand workers reported that the ammonia concentration in *H. pylori* mucus is fourfold greater than in uninfected mucus,[13] almost certainly due to *H. pylori* urease. Nonionic ammonia may cause cellular damage, but only after a rise in pH; which may explain the occasional finding of *H. pylori* on normal mucosa. These workers speculated that their findings explain the transient hypochlorhydria found after voluntary ingestion of *H. pylori*.[14] Vacuolization of tissue culture cells by all strains of *H. pylori* can be seen in the presence of urea; with a urea concentration of 7.5 m*M*, vacuolization was seen in 25% of cells, but, with a urea concentration of 20 m*M*, was seen in 80 to 100% of cells.[15] *H. pylori* gastritis is associated with disruption of intercellular junctions, which would allow plasma urea to leak out. In uremic patients with *H. pylori*, the gastritis is particularly severe.[16] Other workers have also reported that *H. pylori* urease in the presence of urea is toxic to human gastric epithelial cells.[17]

H. pylori lipase and phospholiphase[18,19] are discussed in Chapter 7. They degrade gastric mucosal lipid and reduce mucosal defense. Colloidal bismuth subcitrate causes a 20% reduction in lipase activity and a 60% reduction in phospholipase activity, which may explain part of its action against *H. pylori*.[18]

D. NEUROTRANSMITTERS

Gut infection in the rat causes hypermotility, which has been shown to be mediated by neurotransmitters.[20] Lymphocytic cells are a dominant feature of *H. pylori* gastritis, and lymphocytes have receptors for neurotransmitters.[21] There are genetically determined differences in lymphocyte reactivity, so individuals vary in their responsiveness, and probably also in their neurotransmitter release. Thus, abnormalities of motility may occur only in a genetic subgroup of those with *H. pylori* gastritis.[22] Theoretically, hypermotility could be a factor causing raised pH in the duodenum which is known to lead to gastric metaplasia,[23] but many DU patients exhibit hypomotility. Variable paracrine production of neurotransmitters, especially when *H. pylori* is acquired at an

.y age, may account for the great frequency of asymptomatic gastritis; but ...is may still lead to the later development of carcinoma.[24,25]

E. BASIC PATHOGENETIC PHENOMENA

H. pylori is able to generate paf-acether, a known cause of tissue damage.[26] Also, *H. pylori* synthesizes and secretes FMLP (N-formyl-methionyl-leucyl-phenylalanine) — a chemotactic peptide which stimulates and attracts polymorphonuclear leukocytes;[27] the latter are found in *H. pylori* gastritis. In the gastric mucosa of patients with *H. pylori*, there is a lower histamine concentration than in uninfected mucosa.[28] This is possibly due to increased histamine release, which could in turn induce increased gastric acid secretion. This may be other evidence for paracrine neurotransmitter release. There is a correlation between the concentration of leukotrine B_4 which is higher in the mucosa of infected patients with *H. pylori* than in uninfected mucosa.[29] A dynamic movement of *H. pylori* from areas of damaged epithelium to normal areas may be a repeating cycle.[30]

III. *H. PYLORI* AND DUODENAL ULCER

A more detailed discussion of this subject is found in Chapter 14. The main segments of the cascade that leads to duodenal ulcer have been known since 1988.[3] In islands of gastric metaplasia *H. pylori* from the antrum will cause duodenitis. The inflamed duodenum is ulcerated by the action of acid and pepsin, sometimes with concomitant factors, such as smoking. If *H. pylori* duodenitis is not present, then acid or pepsin, and smoking will not cause ulceration, except in the rare Zollinger-Ellison syndrome; this duodenitis is a stimulus for further gastric metaplasia, thereby increasing the area susceptible to colonization by *H. pylori*, and creating a vicious cycle that culminates in loss of mucosal integrity and repeated ulceration.[31] Gastric metaplasia has been associated with adulthood, male gender, and low fasting gastric juice pH, but not with alcohol, cigarette, or NSAID consumption.[31]

IV. IS GASTRIC CARCINOMA AN INFECTIOUS DISEASE?

This was the title of an article by Correa;[32] the role of *H. pylori* in gastric cancer is fully discussed in Chapters 3 and 6. It is a strong candidate for the previously unknown epidemiological factor characterizing high-risk populations.[33] Excessive use of salt can lead to gastritis which is accompanied by excessive cell replication, and enhances the effectiveness of gastric carcinogens, such as *N*-methyl-*N*^{1}-nitro-*N*-nitrosoguanidine.[32] Polymorphonuclear leukocytes in *H. pylori* gastritis give rise to oxidative bursts, which are known causes of DNA damage and could induce mutations in replicating gastric epithelial cells. On the other hand, antioxidants have a role in preventing DNA damage; fresh fruits and vegetables, which are rich in antioxidants, reduce the

risk of both atrophic gastritis and gastric carcinoma. In the U.S., 1.3% of people with *H. pylori* infection will develop gastric cancer in their lifetime compared to 0.2% of people without *H. pylori*. Parsonnet has estimated that the cost of *H. pylori* screening and treatment would be $25,000 to save 1 year of life.[32a] However, the relative risk of gastric cancer varies in different ethnic groups. In a high gastric cancer risk group such as Japanese Americans, the cost of *H. pylori* screening and treatment would be only $2500 per year of life saved.[32a] Such information is useful for clinicians who wonder whether they should prescribe antibiotics to prevent gastric cancer.

V. ARE SOME STRAINS OF *H. PYLORI* MORE VIRULENT THAN OTHERS?

Detection *in vitro* of differences between strains of *H. pylori* may or may not have relevance to the *in vivo* situation. Some strains after multiple passage in the laboratory may lose some of their virulence factors, such as a soluble hemagglutinin,[34] which they possessed when they were first isolated. The possession of soluble hemagglutinin has been shown to correlate with the ability to form very tight adherence junctions with tissue culture cells, whereas strains without a soluble hemagglutinin do not adhere tightly.[35]

The vacuolating cytotoxin of *H. pylori* was originally detected by Leunk et al.[36] The cytotoxin has been purified and characterized;[36a] the purified, denatured toxin has molecular mass of 87,000 Da, and sera from infected patients recognized the protein. Neutralizing IgG antibodies against the cytotoxin have been detected in 100% of duodenal ulcer patients, but in only 60% of patients with gastritis.[37] This seems to support the work of Figura et al.,[38] who found that 67% of strains from patients with peptic ulcers produced the cytotoxin, whereas only 30% of strains isolated from patients with chronic gastritis produced the cytotoxin. As the development of duodenal ulcer requires the presence of gastric metaplasia in the duodenum, even with a virulent strain of *H. pylori* in the stomach, if gastric metaplasia is not present in the duodenum, then duodenal ulcer will not develop. However, if the gene for the cytotoxin can be identified and cloned, it can be determined how many strains of *H. pylori* have the gene, and whether passage in the laboratory affects expression of the gene. When the urease activity of *H. pylori* is apparently lost, the gene is still present. Certainly, the production of ammonia by *H. pylori* augments the effect of the cytotoxin.

One argument against the significance of the cytotoxin as a virulence factor is that in gnotobiotic piglets successful colonization correlated well with motility of the strains, but not well with cytotoxin production.[89] However, this infection model may not be relevant to the development of duodenal ulcer in humans. A possibly different cytotoxin which causes rounding and damage of Chinese hamster ovary cells, has been detected by Guerrant et al.[40] Urease-negative mutants of *H. pylori* failed to colonize any gnotobiotic piglets, and therefore urease must be an essential virulence factor.[41]

Another difference between *H. pylori* strains *in vivo* and *in vitro* is the thickness of the glycocalyx, which is detected by tannic acid staining.[42] Endoscopic biopsy specimens show an abundant production of this glycocalyx material *in vivo*, but strains cultured on solid media failed to show this glycocalyx, and only strains in shaken broth cultures show a thin layer of the glycocalyx.[42] All strains have shown similar quantities, so there is no evidence that some strains are more virulent than others in this regard. Superficial adhering material has been studied *in vitro* with tissue culture cells, and all strains studied were found to be strongly adherent.[43]

In conclusion, some strains may be more virulent than others, but the evidence at the moment is inconclusive. Loss of virulence factors after multiple *in vitro* passage requires much more study. The reason why only a few people develop DU of the many who have gastritis may be more due to host factors with variable frequency of gastric metaplasia in the duodenum and variable neurotransmitter release. There is a lower limit of peak acid output of 15 mmol/h below which DU does not develop.[44] In areas of Colombia, almost 50% of 30-year-old persons have a high gastric pH,[45] and hypoacidity in young adults is very common in the mountain regions of Chile, Iran, and also in south India and China, which may be associated with a poor diet.[46] Therefore, in such communities, DU would be rare even though gastritis is extremely common.

VI. PRELIMINARY DISEASE ASSOCIATION WITH HYPERTENSION

An unexpected finding was made when 539 patients with dyspepsia were studied in England.[47] Endoscopic biopsy specimens were studied for *H. pylori*. An association was discovered between the presence of *H. pylori* and hypertension defined as diastolic blood pressure consistently in excess of 95 mmHg. Fourteen patients with *H. pylori* (42%) were hypertensive compared with only 6 (12%) patients without *H. pylori*. However, the patients with *H. pylori* were somewhat older than the ones without *H. pylori*. Excessive alcohol consumption was unlikely to be a common pathogenic factor in the patients studied. Whether this information will be confirmed remains to be seen.

VII. THE POSSIBILITY OF AN *H. PYLORI* VACCINE

If a vaccine could be developed which prevented *H pylori* infection, nearly all duodenal ulcers, 60% of gastric cancer, and a high proportion of gastric ulcers would be prevented, together with a vast number of patients with chronic gastritis. However, immunologists are aware that any vaccine may also have some detrimental effects, and at this stage we cannot predict whether the benefits of an *H. pylori* vaccine would outweigh the dangers. The successful development of any bacterial vaccine involves the following major tasks:

1993

1. Identification of humoral and cellular host factors that confer protection.
2. Selection of bacterial strains and epitopes that stimulate such factors and the inclusion of appropriate epitopes into the vaccine.
3. Addition of immunogenic epitopes that ensure T-cell recruitment and omission of suppressor-gene epitopes.
4. Testing of the vaccine in an animal model.
5. Testing of the vaccine in humans.

Task one requires a full understanding of the pathogenesis of *H. pylori* lesions, such as adherence to gastric mucosa; if immunization can prevent adherence, this may be adequate to prevent infection. The selection of appropriate epitopes to include in a successful vaccine and elimination of unwanted epitopes is a sophisticated study which has been undertaken for several bacterial species. For example, with *Vibrio cholerae*, oligosaccharides of surface-exposed lipopolysaccharides (LPS) were shown to be strong bacterial surface immunogens in the mucosal immune system. A sophisticated technique was employed in which anti-LPS hybridoma cells were implanted subcutaneously in syngeneic neonatal mice, which resulted in bodywide secretion of monospecific sIgA via transepithelial transport, mediated by the polymeric Ig receptor. When challenged with a lethal oral dose of *V. cholerae*, all the tumor-bearing animals were consistently protected.[48] This showed that IgA with a single specificity directed against a surface carbohydrate epitope could protect against bacterial infection and disease, presumably by preventing the entry of organisms into the epithelial surface microenvironment, preventing adherence and colonization.[49] For *H. pylori*, protective epitopes must be identified, using similar sophisticated systems. Adherence is a vital preliminary stage in colonization by *H. pylori*. Thus, the discovery that strains possessing a soluble hemagglutinin are the ones that adhere tightly is relevant,[34] and the epitope responsible for the soluble hemagglutinin probably should be included in a vaccine.

Ideally, a vaccine should protect after oral vaccination. Protective antibody production after immunization with peptides in their free form, without the use of carrier proteins, requires the peptides to contain appropriate antibody recognition sites (B-cell epitopes) as well as sites capable of soliciting T-cell help (T_H-cell epitopes) for antibody production.[50] However, efficient targeting of protein or peptide antigens requires that the epitopes are inserted into a carrier molecule which exhibits high affinity for binding sites. The carrier molecules should also contain T-cell epitopes in order to stimulate the helper T cells which are responsible for a secondary immune response. With *Mycobacterium tuberculosis*, some of the immunogenic proteins bear a close homology to the eukaryotic heat-shock proteins, including the 65-kDa heat-shock protein, which appear to be immunodominant.[51] Significantly, a homologue of the human heat-shock protein 60 family has been identified in *H. pylori*,[52] which is urease associated.[52a] This was originally described as a 54-kDa protein which

appeared to be a major, surface-exposed protein in *H. pylori*, and was highly immunogenic. Not only could this protein contribute to gastric injury by stimulating T-cells, which cross-react with similar determinants on stressed host cells, but, if this epitope was included in a vaccine, it could produce undesirable cellular responses. Other epitopes may induce undesirable suppression of cellular responses, such as are responsible for the prolonged carrier state that is characteristic of tuberculosis.[53] The development of effective immunogens against pulmonary tuberculosis may provide a model for an *H. pylori* vaccine. Cloning of putative virulence or protection genes into recombinant carriers that can produce large quantities of the specific immunogen while multiplying within the vaccinated host is being attempted for *M. tuberculosis*.[54] To obtain effective vaccines for human use, it may be necessary to transfer the protective gene or genes to a carrier that is able to establish a persistent self-limiting infection within the vaccinated host, possibly within the lymphoreticular organs.[54] The most important step in this process is the selection of the correct protective gene or genes responsible for the production of protective immunogens.[55] Multiple genes will be involved in this process, of which the most important epitope will be the one that triggers the memory immune T-cell response.[56] Also, selection of a genomic library will have to exclude unwanted suppressor genes.[52] Methods used for selecting these genes in *M. tuberculosis* have depended on the use of monoclonal antibodies or T-cell clones that have the ability to respond to heat-killed *Mycobacteria* or their sonicates.[57] More recent developments have involved the use of a "shuttle phasmid".[58]

Up to the present time, attempts at immunization with *H. pylori* and other helicobacters have been naive, using whole cell bacteria for oral or parenteral inoculation,[59] but oral *H. felis* plus cholera toxin protected mice against challenge with *H. felis*.[59a] Because whole cell vaccines could enhance tissue damage in previously sensitized individuals, only naive persons should be vaccinated. Far more sophisticated approaches are required, such as have been discussed for *M. tuberculosis*;[60] in this disease different immunogens are immunodominant at different stages of the infection, so that multigenic recombinants will be required in a vaccine. Also, fully protective vaccines will require the identification and deletion of genes responsible for humoral or suppressor-cell responses on the part of the vaccinated host. Certainly, any recombinant vaccine must not induce progressive disease in the very young or the immunodeficient patient. Testing of an *H. pylori* vaccine in animal models is now becoming possible and is discussed in Chapter 24. As a preliminary screening method, *H. felis* in the mouse may be a valuable model.[59,61] Such a model can also be used to study the antibacterial effect of single drugs and combinations. Unfortunately, *H. pylori* administered to ferrets, free of *H. mustelae*, failed to colonize the ferrets; but sparse colonization followed oral inoculation of *H. felis* into ferrets.[62] Whether either of the *H. felis* models will extrapolate reliably for *H. pylori* vaccine studies remains to be evaluated. *H. mustelae* may also be

a useful model in ferrets.[63] Especially with regard to adherence mechanisms, the difference between *H. pylori*, *H. felis*, and *H. mustelae* may be critical for investigation of specific epitopes for adherence. In this connection, detailed discussion of the chemical configurations involved is found in Chapter 12. Therefore, the use of chimpanzees and monkeys to test an *H. pylori* vaccine will almost certainly be required, because the *H. pylori* epitopes in the vaccine may not be entirely successful in other models. The rhesus monkey has been successfully inoculated with human *H. pylori* by some workers,[64] but others failed with human *H. pylori* but were successful with rhesus-origin "*H. pylori*".[65]

It has been suggested that with *H. pylori* the main success of the immune response is to prevent the infection from spreading outside the stomach. For a successful vaccine, that is obviously an insufficient objective. In conclusion, a middle course must be steered between naive optimism and unnecessary pessimism. Sophisticated molecular biology and meticulous incorporation of all known facts about pathogenesis should ensure a steady progression and eventual production of an efficient and harmless vaccine.

REFERENCES

1. **Blaser, M. J.,** Hypotheses on the pathogenesis and natural history of *Helicobacter pylori*-induced inflammation, *Gastroenterology*, 102, 720, 1990.
2. **Oi, M., Oshida, K., and Sugimura, S.,** The location of gastric ulcer, *Gastroenterology*, 36, 45, 1959.
3. **Goodwin, C. S.,** Duodenal ulcer, *Campylobacter pylori*, and the "leaking roof" concept, *Lancet*, 31, 1467, 1988.

3a. **Mapstone, N. P., Lynch, D. A. F., Axon, A. T. R., Dixon, M. F., and Quirke, P.,** The detection of *Helicobacter pylori* in faeces by the polymerase chain reaction, *Br. J. Med. Sci.*, 161(suppl. 10), 29, 1992.

3b. **Hopkins, R., Vial, P., Ferreccio, C., Russell, R., and Morris, J. G.,** Increased *Helicobacter* seropositivity among Chilean children who consume uncooked vegetables: support for a water-borne mode of transmission, *Clin. Res.*, 40, 240A, 1992.

4. **Segal, E. D., Shon, J., and Tompkins, L. S.,** Characterization of *Helicobacter pylori* urease mutants, *Infect. Immun.*, 60, 1883, 1992.
5. **Taylor, M. B., Goodwin, C. S., and Karim, Q. N.,** Two urease activities with different pH optima in *Campylobacter pylori* and similar organisms, *FEMS Microbiol. Lett.*, 55, 259, 1988.
6. **Ferrero, R. L. and Lee, A.,** The importance of urease in acid protection for the gastric-colonising bacteria, *Helicobacter pylori* and *Helicobacter felis* sp. nov., *Microbiol. Ecol. Health Dis.*, 4, 121, 1991.
7. **Pruul, H., Goodwin, C. S., McDonald, P. J., Lewis, G., and Pankhurst, D.,** Hydrophobic characterisation of *Helicobacter (Campylobacter) pylori*, *J. Med. Microbiol.*, 32, 93, 1990.
8. **Smith, J. I., Drumm, B., Neumann, A.W., Policova, Z., and Sherman, P. M.,** *In vitro* surface properties of the newly recognized gastric pathogen *Helicobacter pylori*, *Infect. Immun.*, 58, 3056, 1990.

9. **Sidebotham, R. L. and Baron, J. H.,** Hypothesis: *Helicobacter pylori*, urease, mucus, and gastric ulcer, *Lancet*, 335, 193, 1990.
10. **Negrini, R., Lisato, L., Zanella, I., Cavazzini, L., Gullini, S., Villanacci, V., Poiesi, C., Albertini, A., and Ghielmi, S.,** *Helicobacter pylori* infection induces antibodies cross-reacting with human gastric mucosa, *Gastroenterology*, 101, 437, 1991.
11. **Logan, R. P. H., Polson, R. J., Rao, G., Walker, M. M., Pedley, S., Harris, J. R. W., Pinching, A. J., and Baron, J. H.,** *Helicobacter pylori* and HIV infection, *Lancet*, 335, 1456, 1990.
12. **Karttunen, R.,** Blood lymphocyte proliferation, cytokine secretion and appearance of T cells with activation surface markers in cultures with *Helicobacter pylori*. Comparison of the responses of subjects with and without antibodies to *H. pylori*, *Clin. Exp. Immunol.*, 83, 396, 1991.
13. **Thomsen, L., Tasman-Jones, C., Morris, A., Wiggins, P., Less, S., and Forlong, C.,** Ammonia produced by *Campylobacter pylori* neutralizes H^+ moving through gastric mucus, *Scand. J. Gastroenterol.*, 24, 761, 1989.
14. **Morris A. and Nicholson, G.,** Ingestion of *Campylobacter pyloridis* causes gatritis and raised fasting gastric pH, *Am. J. Gastroenterol.*, 82, 192, 1987.
15. **Xu, J. K., Goodwin, C. S., Cooper, M., and Robinson, J.,** Intracellular vacuolization caused by the urease of *Helicobacter pylori*, *J. Infect. Dis.*, 161, 1302, 1990.
16. **Fernandez, D. K., Brady, C. F., and Clement, D. J.,** *Campylobacter pylori* in patients with chronic renal disease, *Lancet*, 96, A148, 1989.
17. **Smoot, D. T., Mobley, H. L. T., Chippendale, G. R., Lewison, J. F., and Resau, J. H.,** *Helicobacter pylori* urease activity is toxic to human gastric epithelial cells, *Infect. Immun.*, 58, 1992, 1990.
18. **Slomiany, B. L., Kasinathan, C., and Slomiany, A.,** Lipolytic activity of *Campylobacter pylori*: effect of colloidal bismuth subcitrate (De-Nol), *Am. J. Gastroenterol.*, 84, 1273, 1989.
19. **Slomiany, B. L., Nishikawa, H., Piotrowski, J., Okazaki, K., and Slomiany, A.,** Lipolytic activity of *Campylobacter pylori*: effect of Sofalcone, *Digestion*, 43, 33, 1989.
20. **Vermillion, D. L. and Collins, S. M.,** Increased responsiveness of jejunal longitudinal muscle in *Trichinella*-infected rats, *Am. J. Physiol.*, 254, G124, 1988.
21. **Stanisz, A. M., Befus, D., and Bienenstock, J.,** Differential aspects of vasoactive intestinal peptide, substance P, and somatostatin on immunoglobulin synthesis and proliferations by lymphocytes from Peyer's patches, mesenteric lymph nodes, and spleen, *J. Immunol.*, 136, 152, 1986.
22. **Goodwin, C. S., Gordon, A., and Burke, V.,** *Helicobacter pylori (Campylobacter pylori)* and duodenal ulcer, *Med. J. Aust.*, 153, 66, 1990.
23. **Wyatt, J. I., Rathbone, B. J., Dixon, M. F., and Heatley, R. V.,** *Campylobacter pyloridis* and acid induced gastric metaplasia in the pathogenesis of duodenitis, *J. Clin. Pathol.*, 40, 841, 1987.
24. **Parsonnet, J., Friedman, G. D., Vandersteen, D. P., Change, Y., Vogelman, J. H., Orentreich, N., and Sibley, R., K.,** *Helicobacter pylori* infection and the risk of gastric carcinoma, *N. Engl. J. Med.*, 325, 1127, 1991.
25. **Nomura, A., Stemmermann, G. N., Chyou, P.-H., Kato, I., Perez-Perez, G. I., and Blaser, M. J.,** *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii, *N. Enlg. J. Med.* 325, 1132, 1991.
26. **Denizot, Y., Sobhani, I., Rambaud, J.-C., Lewin, M., Thomas, Y., and Benveniste, J.,** Paf-acether synthesis by *Helicobacter pylori*, *Gut*, 31, 1242, 1990.
27. **Mooney, C., Keenan, J., Munster, D., Wilson, I., Allardyce, R., Bagshaw, P., Chapman, B., and Chadwick, V.,** Neutrophil activation by *Helicobacter pylori*, *Gut*, 32, 853, 1991.
28. **Queiroz, D. M., Mendes, E. N., Roucha, G. A., Cunha-Melo, J. R., Brabosa, A. J., Lima, G. F., and Liveira, C. A.,** *Helicobacter pylori* and gastric histamine concentrations, *J. Clin. Pathol.*, 44, 612, 1991.
29. **Fukuda, T., Kimura, S., Arakawa, T., and Kobayashi, K.,** Possible role of leukotrienes in gastritis associated with *Campylobacter pylori*, *J. Clin. Gastroenterol.*, 12(suppl.), 131, 1990.

30. **Ormand, J. E. and Talley, N. J.,** *Campylobacter pylori*, mucus, and peptic ulceration, *J. Clin. Gastroenterol.*, 5, 492, 1989.
31. **Wyatt, J. I., Rathbone, B. J., Sobala, G. M., Shallcross, T., Heatley, R. V., Axon, A. T. R., and Dixon, M. F.,** Gastric epithelium in the duodenum: its association with *Helicobacter pylori* and inflammation, *J. Clin. Pathol.*, 43, 981, 1990.
32. **Correa, P.,** Is gastric carcinoma an infectious disease?, *N. Engl. J. Med.*, 325, 1170, 1991.
32a. **Parsonnet, J.,** Potential utility of *H. pylori* screening and treatment in prevention of gastric cancer, *Ir. J. Med. Sci.*, 161(suppl. 10), 25, 1992.
33. **Haenzel, W., Kurihara, M., Segi, M., and Lee, R. K.,** Stomach cancer among Japanese in Hawaii, *J. Natl. Cancer Inst.*, 49, 969, 1972.
34. **Armstrong, J. A., Cooper, M., Goodwin, C. S., Robinson, J., Wee, S. H., Burton, M., and Burke, V.,** Influence of soluble haemagglutinins on adherence of *Helicobacter pylori* to HEp-2 cells, *J. Med. Microbiol.*, 34, 181, 1991.
35. **Robinson, J., Goodwin, C. S., Cooper, M., Burke, V., and Mee, B. J.,** Soluble and cell-associated haemagglutinins of *Helicobacter (Campylobacter) pylori*, *J. Med. Microbiol.*, 33, 277, 1990.
36. **Leunk, R. D., Johnson, P. T., David, B. C., Kraft, W. G., and Morgan, D. R.,** Cytotoxic activity in broth-culture filtrates of *Campylobacter pylori* strains, *J. Clin. Microbiol.*, 26, 93, 1988.
36a. **Cover, T. L. and Blaser, M. J.,** Purification and characterisation of the vacuolating toxin from *Helicobacter pylori*, *J. Biolog. Chem.*, 267, 10570, 1992.
37. **Leunk, R. D., Ferguson, M. A., Morgan, D. R., Low, D. E., and Simor, A. E.,** Antibody to cytotoxin in infection by *Helicobacter pylori*, *J. Clin. Microbiol.*, 28, 1181, 1990.
38. **Figura, N., Guglielmetti, P., Rossolini, A., Barberi, A., Grazia, C., Musmanno, R. A., Russi, M., and Quaranta, S.,** Cytotoxin production by *Campylobacter pylori* strains isolated from patients with peptic ulcers and from patients with chronic gastritis only, *J. Clin. Microbiol.*, 27, 225, 1989.
39. **Eaton, K. A., Morgan, D. R., and Krakowka, S.,** *Campylobacter pylori* virulence factors in gnotobiotic piglets, *Infect. Immun.*, 57, 1119, 1989.
40. **Guerrant, R. L., Barrett, L. J., and Marshall, B. J.,** Cytotoxin production by *Campylobacter pylori*, in *Campylobacter V, Proc. 5th Int. Workshop Campylobacter Infection*, Ruiz-Palacios, G. M., Calva, E., and Ruiz-Palacios, B. R., Eds., Instituto Nacional de la Nutricion, Mexico City, Mexico, 1991, 365.
41. **Eaton, K. A., Brooks, C. L., Morgan, D. R., and Krakowka, S.,** Essential role of urease in pathogenesis of gastritis induced by *Helicobacter pylori* in gnotobiotic piglets, *Infect. Immun.*, 59, 2470, 1991.
42. **Goodwin, C. S., Armstrong, J. A., Chilvers, T., Peters, M., Collins, M. D., Sly, L., McConnell, W., and Harper, W. E. S.,** Transfer of *Campylobacter pylori* and *Campylobacter mustelae* to *Helicobacter* gen. nov. as *Helicobacter pylori* comb. nov. and *Helicobacter mustelae* comb. nov., respectively, *Int. J. Syst. Bacteriol.*, 39, 397, 1989.
43. **Fauchere, J. L. and Blaser, M. J.,** Association of *Helicobacter pylori* with epithelial cells, in *Helicobacter pylori, Gastritis and Peptic Ulcer*, Malfertheiner, P. and Dischuneit, H., Eds., Springer-Verlag, Berlin, 1990, 110.
44. **Baron, J. H.,** An assessment of the augmented histamine test in the diagnosis of peptic ulcer, *Gut*, 4, 243, 1963.
45. **Correa, P., Cuello, C., Duque, E., Burbano, L. C., Garcia, F. T., Bolanos, O., Brown, C., and Haenszel, W.,** Gastric cancer in Colombia. III. Natural history of the precurser lesions, *J. Natl. Cancer Inst.*, 57, 1027, 1976.
46. **Correa, P.,** Epidemiology of gastric cancer and its precurser lesions, in *Gastrointestinal Cancer*, De Cosse, J. and Sherlock, P., Eds., Martinus Nijhoff, Amsterdam, 1982, 119.
47. **Barnes, R. J., Uff, J. S., Dent, J. C., Gear, M. W. L., and Wilkinson, S. P.,** Long-term follow up of patients with gastritis associated with *Helicobacter pylori* infection, *Br. J. Gen. Pract.*, 41, 286, 1991.

48. **Winner, L. S., III, Weltzin, J. J., Mekalanos, J. P., Kraehenbuhl, J. P., and Neutra, M. R.,** A novel *in vivo* system to assess secretory IgA mediated protection against enteric pathogens; transepithelial transport of monoclonal anti-*Vibrio cholerae* IgA from hybridoma tumors, *J. Cell Biol.*, 109, 295a, 1989.
49. **Miller, J. F., Mekalanos, J. J., and Falkow, S.,** Coordinate regulation and sensory transduction in the control of bacterial virulence, *Science*, 243, 916, 1989.
50. **Mitchison, M. A.,** The carrier effect in the secondary response to hapten-protein conjugates, II. Cellular cooperation, *Eur. J. Immunol.*, 1, 18, 1971.
51. **Young, D. B., Ivanyi, J., Cox, J. H., and Lamb, J. R.,** The 65-kDa antigen of mycobacteria — a common bacterial protein?, *Immunol. Today*, 8, 215, 1987.
52. **Dunn, B. E., Roop, R. M., Sung, C.-C., Sharma, S. A., Perez-Perez, G. I., and Blaser, M. J.,** Identification and purification of a cpn60 heat-shock protein homolog from *Helicobacter pylori*, *Infect. Immun.*, 60, 1946, 1992.

52a. **Evans, D. J., Evans, D. G., Engstrand, L., and Graham, D. Y.,** Urease-associated heat shock protein of *Helicobacter pylori*, *Infect. Immun.*, 60, 2125, 1992.

53. **Wallis, R. S., Amir-Tahmasseb, M., and Ellner, J. J.,** Induction of interleukin-1 and tumor necrosis factor by mycobacterial proteins: the monocyte western blot, *Proc. Natl. Acad. Sci. U.S.A.*, 87, 3348, 1990.
54. **Bloom, B. R.,** New approaches to vaccine development, *Rev. Infect. Dis.*, 11(suppl. 2), 460, 1989.
55. **Young, D. B., Mehlert A., Bal, V., Mendez-Samperio, P., Ivanyi, J., and Lamb, J. R.,** Stress proteins and the immune response to mycobacteria-antigens as virulence factors?, *Antonie Van Leeuwenhoek*, 54, 431, 1988.
56. **Orme, I. M. and Collins, F. M.,** Cross protection against nontuberculous mycobacterial infections by *Mycobacterium tuberculosis* memory immune T lymphocytes, *J. Exp. Med.*, 163, 203, 1986.
57. **Young, R. A., Bloom, B. R., Grossinsky, C. M., Ivanyi, J., Thomas, D., and Davis, R. W.,** Dissection of *Mycobacterium tuberculosis* antigens using recombinant DNA, *Proc. Natl. Acad. Sci. U.S.A.*, 82, 2583, 1985.
58. **Jacobs, W. R., Jr., Snapper, S. B., Tuckman, M., and Bloom, B. R.,** Mycobacteriophage vector systems, *Rev. Infect. Dis.*, 11(suppl. 2), 404, 1989.
59. **Czinn, S. J. and Nedrud, J. G.,** Oral immunization against *Helicobacter pylori*, *Infect. Immun.*, 59, 2359, 1991.

59a. **Czinn, S., Cai, A., and Nedrud, J.,** Oral immunization protects germ-free mice against infection from *Helicobacter felis*, *Gastroenterology*, 102, A331, 1992.

60. **Collins, F. M.,** Antituberculosis immunity: new solutions to an old problem, *Rev. Infect. Dis.*, 13, 940, 1991.
61. **Lee, A. L., Fox, J. G., Otto, G., and Murphy, J.,** A small model of human *Helicobacter pylori* active chronic gastritis, *Gastroenterol.* 99, 1315, 1990.
62. **Fox, J. G., Otto, G., Murphy, J. C., Taylor, N. S., and Lee, A.,** Gastric colonization of the ferret with *Helicobacter* species: natural and experimental infections, *Rev. Infec. Dis.*, 13(suppl. 8), 671, 1991.
63. **Fox, J. G., Correa, P., Taylor, N. S., Lee, A., Otto, G., Murphy, J. C., and Rose, R.,** *Helicobacter mustelae*-associated gastritis in ferrets, *Gastrotenterology*, 99, 352, 1990.
64. **Baskerville, A. and Newell, D. G.,** Gastritis associated with experimental *Campylobacter pylori* infection in the Rhesus monkey: a model for the human infection?, in *Campylobacter IV, Proc. IVth Intl. Workshop Campylobacter Infection*, Kaijser, B. and Falsen, E., Eds., University of Göteborg, Göteborg, Sweden, 1988, 438.
65. **Euler, A. R., Zurenko, G. E., Moe, J. B., Ulrich, R. G., and Yagi, Y.,** Evaluation of two monkey species (*Macaca mulatta* and *Macaca fascicularis*) as possible models for human *Helicobacter pylori* disease, *J. Clin. Microbiol.*, 28, 2285, 1990.

INDEX

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